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PDE8: A Novel Target in Airway Smooth Muscle

The ubiquitous second messenger 3',5'-cAMP acts as a key player in the signaling cascades that control many physiological and pathophysiological processes in the lung. The intracellular concentrations of cAMP are determined by its production, which is regulated by G-protein-coupled receptors and adenylyl cyclases (ACs), and its degradation by phosphodiesterases (PDEs), which include 11 family members (PDE1–PDE11) and at least 21 isoforms with different splice variants (1). PDEs hydrolyze cyclic nucleotides (cAMP and cGMP) to inactivate 5' monophosphates within subcellular microdomains, thereby modulating cyclic nucleotide signaling pathways. PDE4, PDE7, and PDE8 are cAMP specific, whereas PDE5, PDE6, and PDE9 are cGMP specific. The remaining family members can degrade both cyclic nucleotides, although they prefer one or the other to varying degrees.

PDE4, the most extensively studied cAMP-specific PDE, is highly expressed in airway smooth muscle (ASM) cells. Many studies have provided important information regarding the functions of PDE4 in ASM cells, especially in regulating cell proliferation, contraction, and migration (2–5). A-kinase anchoring proteins (AKAPs) also contribute to spatial and temporal cAMP dynamics by binding directly to protein kinase A (PKA) and its target proteins, thus physically tethering these multiprotein complexes to specific locations in the cells (6). It has been demonstrated that gravin (AKAP250) is able to tether PKA and PDE4D to β_2 -adrenoceptor (β_2 -AR) at the plasma membrane in human embryonic kidney cells, highlighting the importance of compartmentalized cAMP signaling (7). Inhibition of another cAMP-specific enzyme, PDE7, is also believed to induce a variety of cellular effects due to increased cAMP accumulation. BRL50481, a PDE7-selective inhibitor, can relax the airways after histamine-induced contraction in the ovalbumin-sensitized guinea pig model (8). Moreover, the inhibition of PDE7 augments the inhibitory effect of other cAMP-elevating drugs on proinflammatory cells without having such an effect itself (9). However, surprisingly, the role and location of PDE8, a less widely expressed PDE member that has greater cAMP affinity than PDE4, have not yet been studied in ASM cells.

In this issue of the *Journal*, Johnstone and colleagues (pp. 530–541) demonstrate for the first time the transcript, protein, and functional presence of PDE8 in human ASM cells and report on a functional β_2 -AR-AC6-PDE8 signalosome expressed in caveolae (10). To study the functional role of PDE8, they used dipyrindamole, a semiselective PDE5/8 inhibitor, to enhance cAMP accumulation after forskolin stimulation in human ASM cells overexpressing either AC2 or AC6. They found that increased cAMP levels were restricted to ASM cells overexpressing AC6. The authors confirmed this finding by using shRNA knockdown of PDE8A, which indicated that PDE8A was able to hydrolyze cAMP specifically when induced by AC6, with little interference on AC2-induced cAMP generation. Most intriguingly, their study revealed that PDE8 inhibition selectively increased cAMP levels that were generated in response to β_2 -AR stimulation, but had no

effect when E-prostanoid 2 (EP₂) or EP₄ receptors were activated. Thus, PDE8 inhibition, together with β_2 -AR stimulation, reduced serum-induced human ASM cell proliferation, whereas PDE8 inhibition had no such effect on prostaglandin E₂. Modulation of PDE8 activity in human ASM cells may enhance the effect of β_2 -AR signaling on bronchodilation as well, but this needs to be further established in future studies.

Nowadays, several cAMP biosensors are used to visualize cAMP fluctuations in living cells with high temporal and spatial resolution (11). After almost two decades, the fluorescence resonance energy transfer-based cAMP sensors are well developed and widely used to study cAMP dynamics. To monitor cAMP dynamics in the present study, Johnstone and colleagues used another novel genetically encoded cAMP biosensor, named cAMP Difference Detector *in situ* (cADDiS), which employs a much easier standard fluorescent plate reader (10). In contrast to the classical two-fluorophore fluorescence resonance energy transfer-based cAMP biosensor, cADDiS is composed of one circularly permuted GFP and a cAMP-binding domain of an exchange protein directly activated by cAMP (EPAC) 2, which makes it possible to be paired with other colored sensors for multiplex recordings, such as red Ca²⁺ sensors (12). Using this sensor, the authors were able to show that the PDE8-selective inhibitor PF-04957325 had strikingly large effects on cAMP in ASM, indicating that PDE8 activity is at least as important as PDE4 in modulating cAMP dynamics in human ASM cells. Therefore, PDE8 may have therapeutic value in respiratory diseases such as asthma and chronic obstructive pulmonary disease.

Several intriguing questions arise from this study that require further investigation. cAMP has been shown to regulate the ASM contractile state, secretion of inflammatory cytokines and chemokines, and cell proliferation and migration (13). Therefore, it is important to examine the effect of PDE8 inhibition on these responses in further detail in both *in vitro* and *in vivo* experimental models. Moreover, AKAPs, as some of the most important elements in cAMP compartments, play a vital role in modulating cAMP spatial and temporal dynamics. Thus, it is rational to explore the role of different AKAP members in controlling PDE8 activity and localization. The present study shows the colocalization of PDE8 with β_2 -AR-AC6 in caveolae.

In conclusion, this study by Johnstone and colleagues (10) reveals for the first time the selective role of PDE8 in limiting β_2 -AR-AC6-mediated cAMP signaling in human ASM cells, and motivates further exploration of the functional outcomes of PDE8 inhibition. As one of the most predominant cAMP-hydrolyzing PDEs in human ASM cells, PDE8 seems to be a therapeutic target worth pursuing in respiratory diseases. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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